Heterozygous *TYROBP* deletion ($PLOSL_{FIN}$) is not a strong risk factor for cognitive impairment

Karri Kaivola*[a], Lilja Jansson[a], Elmo Saarentaus[a,] Anna Kiviharju[a], Ville Rantalainen[b], Johan G. Eriksson[c,d,e], TE Strandberg[f,g], Tuomo Polvikoski[h], Liisa Myllykangas[i], Pentti J. Tienari[a]

[a]Molecular Neurology, Research Programs Unit, University of Helsinki and Department of Neurology, Helsinki University Hospital, Helsinki, Finland

[b]Department of Psychology and Logopedics, University of Helsinki, Helsinki, Finland

[c]Folkhälsan Research Center, Helsinki, Finland

[d]Department of General Practice and Primary Health Care, University of Helsinki and Helsinki University Hospital, Unit of General Practice, Helsinki, Finland

[e]National Institute for Health and Welfare, Helsinki ,Finland

[f]Centre for Life Course Health Research, University of Oulu, Oulu, Finland

[g]University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[h]Institute of Neuroscience, Newcastle University, UK

[i]Pathology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

*Corresponding author at: Research Programs Unit, Haartmaninkatu 8, P.O. Box 63 00014 University of Helsinki Finland. Tel. +358505223563 E-mail address: karri.kaivola@helsinki.fi

Key words: Genetics; Alzheimer's disease; Dementia; *TYROBP*; *DAP12*; PLOSL

Abstract

Biallelic loss-of-function mutations in *TYROBP* and *TREM2* cause a rare disease that resembles early-onset frontotemporal dementia with bone lesions (PLOSL). Some PLOSL-causing variants in *TREM2* have also been associated with Alzheimer's disease when heterozygous. Here, we studied the $PLOSL_{FIN}$ *TYROBP* deletion that covers four of the gene's five exons. We genotyped 3220 older Finns (mean age 79, range 58-104) and found 11 deletion

carriers (mean age 78, range 60-94). The carrier prevalence was 0.0034 (1 in 293) that matches previous findings in younger cohorts suggesting no significant early mortality. By comparing MMSE scores and diagnoses of dementia we did not find any significant differences between *TYROBP* deletion carriers and non-carriers (all p-values >0.5). Neuropathological analysis of two deletion carriers (aged 89 and 94) demonstrated only minimal beta-amyloid pathology (CERAD score 0). Collectively these results suggest that heterozygous carriership of the *TYROBP* deletion is not a major risk factor of cognitive impairment.

Highlights: 3-5 bullet points

- Loss-of-function mutations in *TYROBP* and *TREM2* cause early-onset frontotemporal dementia with bone lesions (PLOSL)
- *TREM2* and *TYROBP* are part of the same receptor-signaling complex
- Heterozygous variants in *TREM2* are risk factors for neurodegenerative diseases
- The $PLOSL_{FIN}$ deletion of *TYROBP* is not a strong risk factor for cognitive impairment when heterozygous

Keywords

Genetics; Alzheimer's disease; Dementia; *TYROBP*; *DAP12*; PLOSL

1. Introduction

Triggering receptor expressed on myeloid cells 2 (*TREM2*) and TYRO protein tyrosine kinase binding protein (*TYROBP*) are part of the same receptor-signaling complex (Paloneva et al., 2002). Biallelic loss-of-function mutations in *TYROBP* or *TREM2* genes cause polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL, Nasu-Hakola disease) (Paloneva et al., 2000, Paloneva et al., 2002), a rare disease characterized by pathologic fractures, personality changes and presenile dementia (Hakola, 1972). In *TYROBP*, also known as *DAP12*, compound

heterozygous mutations have been reported to cause PLOSL (Kuroda et al., 2007) whereas compound heterozygous mutations in *TREM2* also lead to a frontotemporal dementia –like phenotype (Guerreiro et al., 2013). *TREM2* and *TYROBP* have also been genetically or functionally associated with Alzheimer's disease (AD), both late-onset (LOAD) (Jonsson et al., 2013, Sims et al., 2017, Zhang et al., 2013) and early-onset forms (Pottier et al., 2016, Slattery et al., 2014). While the association of *TREM2* and AD seems strong, less data on *TYROBP* is available and it is inconclusive. However, a recent gene-regulatory network analysis of postmortem brains suggested *TYROBP* as an important regulator of gene expression in Alzheimer's disease (Zhang et al., 2013). In a cohort of 103 Turkish patients with different forms of dementia (average age of onset <66.7 years) a *TYROBP* p.Val55Leu variant was found in three individuals, but this variant was not considered pathogenic (Darwent et al., 2017). In this article, we studied the effect of $PLOSL_{FIN}$-deletion of *TYROBP* on cognitive impairment.

In this study, we genotyped 3220 older Finns for the 5.3kb deletion in *TYROBP* that causes PLOSL in individuals of Finnish ancestry (Paloneva et al., 2000) and found no association with cognitive impairment.

2. Material and Methods

2.1. Cohorts

We studied four population-derived cohorts from the Finnish capital region (Helsinki area) from whom the 5.3kb *TYROBP* $PLOSL_{FIN}$ -deletion was genotyped and information on cognition was available. We also analyzed *APOE* alleles and *APP* p.Ala673Thr variant to compare the background genetic risk burden for cognitive impairment between *TYROPB* deletion carriers and non-carriers. *APOE* ε4 is the most prevalent genetic risk factor for cognitive impairment and *APP* p.Ala673Thr is

a strong protective mutation against Alzheimer's disease and age-associated cognitive decline (Jonsson et al., 2012, Kero et al., 2013).

The Helsinki Birth cohort study (HBCS) consists of 8760 individuals born in Helsinki in 1934-1944 (Barker et al., 2005, Eriksson et al., 2006). In 2001-2004, a random sample of 2003 individuals participated in a clinical study including DNA extraction (Kajantie et al., 2012, Yliharsila et al., 2007) and a random subsample's cognition was assessed with MMSE and CERAD. In addition, registry information up till December 31 of 2013 were checked for dementia hospitalizations and dementia deaths. This included data on diagnoses of any organic dementias, given by physicians in inpatient (1969-2013) and outpatient (1998-2013) care (codes 290.00-290.10 from International Classification of Diseases (ICD)-8, 290, 2912A, 2928C, 2941A, 3310A and 3311A from ICD-9, and F00, F01, F03, F051 and G30 from ICD-10) and of Alzheimer's disease (codes 331.0 and 290.1 from ICD-9, and G30 and F00 from ICD-10) until December 31 of 2013 were derived from the Finnish Hospital Discharge and Causes of Death Registers (Lahti et al., 2014).

Helsinki Businessmen Study (HBS) originally consists of 3,490 healthy Finnish men, born 1919-1934. All participants were businessmen or executives with similar and high socioeconomic status. In 2002-2003, 672 individuals who lived at home were randomly selected for analyses and 650 of them gave a venous blood sample. Participants' cognition was tested using the MMSE (Strandberg et al., 2016). In 2014 cognition was evaluated with a questionnaire.

The DEBATE cohort consists of a random sample of 4,800 individuals living in Helsinki, Finland. In 2000, 400 home-living individuals with stable cardiovascular disease (coronary artery disease, stroke or transient ischemic attack, peripheral artery disease) were clinically examined and cognitive functions were assessed with MMSE. (Uusvaara et al., 2013) In 2014 dementia diagnoses were screened from death certificates.

Vantaa 85+ Study includes 601 individuals, aged 85 years and older, who were living in the city of Vantaa, Finland. 553 individuals underwent neurological examination including MMSE by two neurologists in 1991-1992, with follow-up studies in 1994, 1996, 1999 and 2001. Neuropathological autopsy was performed on 281 individuals (Polvikoski et al., 1995).

The study was approved by the Coordinating Ethics Committee of the Helsinki University Central Hospital. The Vantaa 85+ study was also approved by the Ethics Committee of the Health Centre of the City of Vantaa.

2.2. Genotyping

Genomic DNA was available of 3260 samples from the four different cohorts. Samples from VV85+ were whole genome amplified DNA derived from peripheral blood leukocytes (PBLs). DNA from other samples was derived from PBLs. We used PCR assay with one forward and two reverse primers to genotype the samples. Samples without the deletion produce one amplicon (~500bp), samples heterozygous for the deletion produce two amplicons(~500bp and ~700bp) and samples homozygous for the deletion produce only the larger amplicon (~700bp). Primer sequences were 5' GGCCACATCCGTATGACTG 3' for forward primer, 5' TAGTATGTCCAGTCTCGAGTTCTCA 3' for reverse primer 1 and 5' CTAGTCTGGGCGTGCATTC 3' for reverse primer 2. We used a positive control in all PCR assays.

We performed PCR in 20 µl reactions containing 100ng of DNA, 0.50 µl of Dynazyme II DNA polymerase (Thermo Scientific, Waltham, USA), 1.0 M Betaine (Affymetrix, Santa Clara, USA), 0.3 mM of dNTP (Thermo Scientific), 0.5 mM of each primer (Sigma-Aldrich, St.Louis, USA) and 1.0x optimized Dynazyme PCR buffer (Thermo scientific, Waltham, USA). PCR was started with an initial denaturation at 94˚for 12 min; followed by 40 cycles of 30 sec at 94˚, 15 sec at 60˚ (-1˚ for

every cycle until at 55˚) and 30 sec at 72˚; and a final extension at 72˚ for 10 min. Amplicons were visualized in 2% agarose gel electrophoresis with ethidium bromide to determine the genotype of every sample.

APOE was genotyped as previously described (Myllykangas et al., 1999). *APP* p.Ala673Thr was genotyped as previously described (Kero et al., 2013).

2.3. Assessment of cognitive impairment

In the first analysis, we analyzed MMSE scores as a dichotomized variable where people with an MMSE score ≤24 were considered to have cognitive impairment. In the second analysis, MMSE scores were treated as a continuous variable. In a third analysis, we used cohort-specific information as complementary information. This information was, depending on cohort, a clinical diagnosis of dementia, any dementia diagnosis on death certificate, a questionnaire study with self-reported diagnoses and medications or hospital discharge and cause of death register information on any dementia diagnosis. In this third analysis, individuals with MMSE scores ≤24 or who had any diagnosis of dementia were considered to have cognitive impairment.

2.4. Statistical analyses

Analyses were conducted with SPSS v.24.0 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). We used Fisher's exact test and Mann-Whitney U test to test for the association between cognitive impairment and the deletion. We used the same tests to study the differences in age, sex, *APOE* ε4 and *APP* p.Ala673Thr between deletion carriers and non-carriers.

Statistical power was calculated with the Genetic Power Calculator (http://zzz.bwh.harvard.edu/gpc/, accessed August 10, 2017) (Purcell et al., 2003). Assuming deletion prevalence of 0.0034 based on our sequencing results, 20% prevalence of cognitive impairment in ≥75 years old Finnish population (Rahkonen et al., 2003), relative risk of 2.5, case-

control ratio of 4 and α=0.05, 423 cases were required for 80% power. This number of cases was achieved in all analyses.

3. Results

All cohorts together, we genotyped successfully 3220 (98.7%) individuals and 11 were heterozygous for the deletion, giving a total frequency of 0.0034. DEBATE was the only cohort with no deletion carriers (Supplementary Table1). No sample was homozygous for the deletion as could be expected. A summary of the characteristics of the 11 deletion carriers is shown in Supplementary Table2.

MMSE scores were available for 2390 individuals, including seven deletion carriers. In the first analysis, 497 non-carriers (21%) and one deletion carrier (14%) had cognitive impairment (MMSE ≤24) (Fisher's exact test p=1). When MMSE scores were analyzed as a continuous variable, there was no significant difference between deletion carriers and non-carriers (Mann-Whitney U test p=0.68). In the third analysis, any information on cognition (MMSE, questionnaire, diagnosis of dementia) was retrieved from 3192 individuals including all 11 deletion carriers. Of the deletion carriers, three (27%) and of the non-carriers 692 (22%) had cognitive impairment (Fisher's exact test p=0.71). In all three analyses there were no statistically significant differences between carriers and non-carriers in age, sex, *APP* p.Ala673Thr or *APOE* ε4 status. In the first and second analyses, deletion carriers vs. non-carriers had the following characteristics: mean age 77,9 vs. 76,1 years (mean ranks 1350 vs 1195, p=0.55), 57.1 % vs. 44,9% females (p=0.71) and 28.6 % vs. 32,4 % had the APOE ε4 allele (p=1.0). In the third analysis deletion carriers vs. non-carriers had the following characteristics: mean age 77.6 vs. 78,9 years (mean ranks 1463 vs 1596 p=0.63), 54.5 % vs. 46,7 % females (p=0.76) and 45.5% vs. 32.9 % (p=0.52) . There were 23 (0.7%) carriers of the *APP* p.Ala673Thr variant, none among the *TYROBP* deletion carriers (p=1.0 in all analyses).

Two subjects of the Vantaa85+ study were autopsied at the ages of 89 and 94.Their mean percentage of the cortex covered by methenamine silver-positive senile plaques in sections cut from 4 neocortical samples (neocortical beta-amyloid percentages) were 0% and m 0.18%, both had a CERAD score of 0 and Braak stages 3 and 4. These neuropathological features do not show significant signs of AD pathology. The mean beta-amyloid percentages in the Vantaa85+ population were 3.4%, Braak stage 3.3 and CERAD 1.4 (in the Vantaa 85+ study: score 1= sparse neuritic senile plaques, 2 = moderate frequency of neuritic senile plaques, 3= frequent neuritic senile plaques).

We performed logistic regression analyses to study the effect of *APOE* ε2 and ε4 carriership on cognitive impairment and used age and sex as covariates. *APOE* ε4 carriership associated with cognitive impairment in both MMSE score -based analysis ($p=2.3 \times 10^{-4}$, OR 1.7 (1.3-2.3)) and when all information available on cognition was used ($p=2.4 \times 10^{-9}$, OR=1.9 (1.5-2.4)). *APOE* ε2 associated with better cognition when using cognitive impairment status derived from MMSE scores (p=0.019, OR=0.6 (0.39-0.92)) and showed a trend for better cognition when all information available on cognition was used (p=0.07, OR=0.74 (0.54-1.03)).

4. Discussion

We used MMSE scores and dementia diagnoses to study cognitive impairment on $PLOSL_{FIN}$ deletion carriers and non-carriers and found no statistically significant differences. Neuropathological data on two deletion carriers brings additional support that the deletion is not a major risk factor for Alzheimer's disease. On the contrary, both had very low neocortical beta-amyloid percentages and CERAD scores, which is noteworthy because it is known that *TREM2* and *TYROBP* expressing microglia accumulate around senile plaques but whether the effect is beneficiary or harmful is still under debate.

To the best of our knowledge, we report the first data on cognition and neuropathological examination of older individuals with a heterozygous PLOSL-causing *TYROBP* variant. Our results suggest that the deletion is not a major risk factor for cognitive impairment. The main limitations of our study arise from the rarity of the $PLOSL_{FIN}$ –deletion and larger studies are needed to exclude minor effects on cognition. Moreover, it is not known how much TYROBP protein levels decrease in the deletion carriers since we were not able to study the expression level of TYROBP.

High socioeconomic status is also known to be a protective factor against cognitive impairment and MMSE test may lose some of its sensitivity with highly-educated people. Unfortunately, we did not know the socioeconomic status of most of the participants and could not include the effect of socioeconomic status into our analyses.

The VV85+ samples were whole-genome amplified DNA whereas other samples were genomic DNA extracted from blood. Amplification is a potential source of error when studying larger deletions. In our study, however, the frequency of the studied deletion in the VV85+ samples was in line with the other cohorts suggesting no error occurred during whole genome amplification.

References

Barker, D.J., Osmond, C., Forsen, T.J., Kajantie, E., Eriksson, J.G., 2005. Trajectories of growth among children who have coronary events as adults. N.Engl.J.Med. 353, 1802-1809.

Darwent, L., Carmona, S., Lohmann, E., Guven, G., Kun-Rodrigues, C., Bilgic, B., Hanagasi, H., Gurvit, H., Erginel-Unaltuna, N., Pak, M., Hardy, J., Singleton, A., Bras, J., Guerreiro, R., 2017. Mutations in TYROBP are not a common cause of dementia in a Turkish cohort. Neurobiol.Aging. 58, 240.e1-240.e3.

Eriksson, J.G., Osmond, C., Kajantie, E., Forsen, T.J., Barker, D.J., 2006. Patterns of growth among children who later develop type 2 diabetes or its risk factors. Diabetologia. 49, 2853-2858.

Guerreiro, R., Bilgic, B., Guven, G., Bras, J., Rohrer, J., Lohmann, E., Hanagasi, H., Gurvit, H., Emre, M., 2013. Novel compound heterozygous mutation in TREM2 found in a Turkish frontotemporal dementia-like family. Neurobiol.Aging. 34, 2890.e1-2890.e5.

Hakola, H.P., 1972. Neuropsychiatric and genetic aspects of a new hereditary disease characterized by progressive dementia and lipomembranous polycystic osteodysplasia. Acta Psychiatr.Scand.Suppl. 232, 1-173.

Jonsson, T., Atwal, J.K., Steinberg, S., Snaedal, J., Jonsson, P.V., Bjornsson, S., Stefansson, H., Sulem, P., Gudbjartsson, D., Maloney, J., Hoyte, K., Gustafson, A., Liu, Y., Lu, Y., Bhangale, T., Graham, R.R., Huttenlocher, J., Bjornsdottir, G., Andreassen, O.A., Jonsson, E.G., Palotie, A., Behrens, T.W., Magnusson, O.T., Kong, A., Thorsteinsdottir, U., Watts, R.J., Stefansson, K., 2012. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature. 488, 96-99.

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P.V., Snaedal, J., Bjornsson, S., Huttenlocher, J., Levey, A.I., Lah, J.J., Rujescu, D., Hampel, H., Giegling, I., Andreassen, O.A., Engedal, K., Ulstein, I., Djurovic, S., Ibrahim-Verbaas, C., Hofman, A., Ikram, M.A., van Duijn, C.M., Thorsteinsdottir, U., Kong, A., Stefansson, K., 2013. Variant of TREM2 associated with the risk of Alzheimer's disease. N.Engl.J.Med. 368, 107-116.

Kajantie, E., Pietilainen, K.H., Wehkalampi, K., Kananen, L., Raikkonen, K., Rissanen, A., Hovi, P., Kaprio, J., Andersson, S., Eriksson, J.G., Hovatta, I., 2012. No association between body size at birth and leucocyte telomere length in adult life--evidence from three cohort studies. Int.J.Epidemiol. 41, 1400-1408.

Kero, M., Paetau, A., Polvikoski, T., Tanskanen, M., Sulkava, R., Jansson, L., Myllykangas, L., Tienari, P.J., 2013. Amyloid precursor protein (APP) A673T mutation in the elderly Finnish population. Neurobiol.Aging. 34, 1518.e1-1518.e3.

Kuroda, R., Satoh, J., Yamamura, T., Anezaki, T., Terada, T., Yamazaki, K., Obi, T., Mizoguchi, K., 2007. A novel compound heterozygous mutation in the DAP12 gene in a patient with Nasu-Hakola disease. J.Neurol.Sci. 252, 88-91.

Lahti, M., Eriksson, J.G., Heinonen, K., Kajantie, E., Lahti, J., Wahlbeck, K., Tuovinen, S., Pesonen, A.K., Mikkonen, M., Osmond, C., Raikkonen, K., 2014. Maternal Grand Multiparity and the Risk of Severe Mental Disorders in Adult Offspring. PLoS One. 9, e114679.

Myllykangas, L., Polvikoski, T., Sulkava, R., Verkkoniemi, A., Crook, R., Tienari, P.J., Pusa, A.K., Niinisto, L., O'Brien, P., Kontula, K., Hardy, J., Haltia, M., Perez-Tur, J., 1999. Genetic association of alpha2-macroglobulin with Alzheimer's disease in a Finnish elderly population. Ann.Neurol. 46, 382-390.

Paloneva, J., Kestila, M., Wu, J., Salminen, A., Bohling, T., Ruotsalainen, V., Hakola, P., Bakker, A.B., Phillips, J.H., Pekkarinen, P., Lanier, L.L., Timonen, T., Peltonen, L., 2000. Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. Nat.Genet. 25, 357-361.

Paloneva, J., Manninen, T., Christman, G., Hovanes, K., Mandelin, J., Adolfsson, R., Bianchin, M., Bird, T., Miranda, R., Salmaggi, A., Tranebjaerg, L., Konttinen, Y., Peltonen, L., 2002. Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. Am.J.Hum.Genet. 71, 656-662.

Polvikoski, T., Sulkava, R., Haltia, M., Kainulainen, K., Vuorio, A., Verkkoniemi, A., Niinisto, L., Halonen, P., Kontula, K., 1995. Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. N.Engl.J.Med. 333, 1242-1247.

Pottier, C., Ravenscroft, T.A., Brown, P.H., Finch, N.A., Baker, M., Parsons, M., Asmann, Y.W., Ren, Y., Christopher, E., Levitch, D., van Blitterswijk, M., Cruchaga, C., Campion, D., Nicolas, G., Richard, A.C., Guerreiro, R., Bras, J.T., Zuchner, S., Gonzalez, M.A., Bu, G., Younkin, S., Knopman, D.S., Josephs, K.A., Parisi, J.E., Petersen, R.C., Ertekin-Taner, N., Graff-Radford, N.R., Boeve, B.F., Dickson, D.W., Rademakers, R., 2016. TYROBP genetic variants in early-onset Alzheimer's disease. Neurobiol.Aging. 48, 222.e9-222.e15.

Purcell, S., Cherny, S.S., Sham, P.C., 2003. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics. 19, 149-150.

Rahkonen, T., Eloniemi-Sulkava, U., Rissanen, S., Vatanen, A., Viramo, P., Sulkava, R., 2003. Dementia with Lewy bodies according to the consensus criteria in a general population aged 75 years or older. J.Neurol.Neurosurg.Psychiatry. 74, 720-724.

Sims, R., van der Lee, S.J., Naj, A.C., Bellenguez, C., Badarinarayan, N., Jakobsdottir, J., Kunkle, B.W., Boland, A., Raybould, R., Bis, J.C., Martin, E.R., Grenier-Boley, B., Heilmann-Heimbach, S., Chouraki, V., Kuzma, A.B., Sleegers, K., Vronskaya, M., Ruiz, A., Graham, R.R., Olaso, R., Hoffmann, P., Grove, M.L., Vardarajan, B.N., Hiltunen, M., Nothen, M.M., White, C.C., Hamilton-Nelson, K.L., Epelbaum, J., Maier, W., Choi, S.H., Beecham, G.W., Dulary, C., Herms, S., Smith, A.V., Funk, C.C., Derbois, C., Forstner, A.J., Ahmad, S., Li, H., Bacq, D., Harold, D., Satizabal, C.L., Valladares, O., Squassina, A., Thomas, R., Brody, J.A., Qu, L., Sanchez-Juan, P., Morgan, T., Wolters, F.J., Zhao, Y., Garcia, F.S., Denning, N., Fornage, M., Malamon, J., Naranjo, M.C.D., Majounie, E., Mosley, T.H., Dombroski, B., Wallon, D., Lupton, M.K., Dupuis, J., Whitehead, P., Fratiglioni, L., Medway, C., Jian, X., Mukherjee, S., Keller, L., Brown, K., Lin, H., Cantwell, L.B., Panza, F., McGuinness, B., Moreno-Grau, S., Burgess, J.D., Solfrizzi, V., Proitsi, P., Adams, H.H., Allen, M., Seripa, D., Pastor, P., Cupples, L.A., Price, N.D., Hannequin, D., Frank-Garcia, A., Levy, D., Chakrabarty, P., Caffarra, P., Giegling, I., Beiser, A.S., Giedraitis, V., Hampel, H., Garcia, M.E., Wang, X., Lannfelt, L., Mecocci, P., Eiriksdottir, G., Crane, P.K., Pasquier, F., Boccardi, V., Henandez, I., Barber, R.C., Scherer, M., Tarraga, L., Adams, P.M., Leber, M., Chen, Y., Albert, M.S., Riedel-Heller, S., Emilsson, V., Beekly, D., Braae, A., Schmidt, R., Blacker, D., Masullo, C., Schmidt, H., Doody, R.S., Spalletta, G., Jr, W.T.L., Fairchild, T.J., Bossu, P., Lopez, O.L., Frosch, M.P., Sacchinelli, E., Ghetti, B., Yang, Q., Huebinger, R.M., Jessen, F., Li, S., Kamboh, M.I., Morris, J., Sotolongo-Grau, O., Katz, M.J., Corcoran, C., Dunstan, M., Braddel, A., Thomas, C., Meggy, A., Marshall, R., Gerrish, A., Chapman, J., Aguilar, M., Taylor, S., Hill, M., Fairen, M.D., Hodges, A., Vellas, B., Soininen, H., Kloszewska, I., Daniilidou, M., Uphill, J., Patel, Y., Hughes, J.T., Lord, J., Turton, J., Hartmann, A.M., Cecchetti, R., Fenoglio, C., Serpente, M., Arcaro, M., Caltagirone, C., Orfei, M.D., Ciaramella, A., Pichler, S., Mayhaus, M., Gu, W., Lleo, A., Fortea, J., Blesa, R., Barber, I.S., Brookes, K., Cupidi, C., Maletta, R.G., Carrell, D., Sorbi, S., Moebus, S., Urbano, M., Pilotto, A., Kornhuber, J., Bosco, P., Todd, S., Craig, D., Johnston, J., Gill, M., Lawlor, B., Lynch, A., Fox, N.C., Hardy, J., ARUK Consortium, Albin, R.L., Apostolova, L.G., Arnold, S.E., Asthana, S., Atwood, C.S., Baldwin, C.T., Barnes, L.L., Barral, S., Beach, T.G., Becker, J.T., Bigio, E.H., Bird, T.D., Boeve, B.F., Bowen, J.D., Boxer, A., Burke, J.R., Burns, J.M., Buxbaum, J.D., Cairns, N.J., Cao, C., Carlson, C.S., Carlsson, C.M., Carney, R.M., Carrasquillo, M.M., Carroll, S.L., Diaz, C.C., Chui, H.C., Clark, D.G., Cribbs, D.H., Crocco, E.A., DeCarli, C., Dick, M., Duara, R., Evans, D.A., Faber, K.M., Fallon, K.B., Fardo, D.W., Farlow, M.R., Ferris, S., Foroud, T.M., Galasko, D.R., Gearing, M., Geschwind, D.H., Gilbert, J.R., Graff-Radford, N.R.,

Green, R.C., Growdon, J.H., Hamilton, R.L., Harrell, L.E., Honig, L.S., Huentelman, M.J., Hulette, C.M., Hyman, B.T., Jarvik, G.P., Abner, E., Jin, L.W., Jun, G., Karydas, A., Kaye, J.A., Kim, R., Kowall, N.W., Kramer, J.H., LaFerla, F.M., Lah, J.J., Leverenz, J.B., Levey, A.I., Li, G., Lieberman, A.P., Lunetta, K.L., Lyketsos, C.G., Marson, D.C., Martiniuk, F., Mash, D.C., Masliah, E., McCormick, W.C., McCurry, S.M., McDavid, A.N., McKee, A.C., Mesulam, M., Miller, B.L., Miller, C.A., Miller, J.W., Morris, J.C., Murrell, J.R., Myers, A.J., O'Bryant, S., Olichney, J.M., Pankratz, V.S., Parisi, J.E., Paulson, H.L., Perry, W., Peskind, E., Pierce, A., Poon, W.W., Potter, H., Quinn, J.F., Raj, A., Raskind, M., Reisberg, B., Reitz, C., Ringman, J.M., Roberson, E.D., Rogaeva, E., Rosen, H.J., Rosenberg, R.N., Sager, M.A., Saykin, A.J., Schneider, J.A., Schneider, L.S., Seeley, W.W., Smith, A.G., Sonnen, J.A., Spina, S., Stern, R.A., Swerdlow, R.H., Tanzi, R.E., Thornton-Wells, T.A., Trojanowski, J.Q., Troncoso, J.C., Van Deerlin, V.M., Van Eldik, L.J., Vinters, H.V., Vonsattel, J.P., Weintraub, S., Welsh-Bohmer, K.A., Wilhelmsen, K.C., Williamson, J., Wingo, T.S., Woltjer, R.L., Wright, C.B., Yu, C.E., Yu, L., Garzia, F., Golamaully, F., Septier, G., Engelborghs, S., Vandenberghe, R., De Deyn, P.P., Fernadez, C.M., Benito, Y.A., Thonberg, H., Forsell, C., Lilius, L., Kinhult-Stahlbom, A., Kilander, L., Brundin, R., Concari, L., Helisalmi, S., Koivisto, A.M., Haapasalo, A., Dermecourt, V., Fievet, N., Hanon, O., Dufouil, C., Brice, A., Ritchie, K., Dubois, B., Himali, J.J., Keene, C.D., Tschanz, J., Fitzpatrick, A.L., Kukull, W.A., Norton, M., Aspelund, T., Larson, E.B., Munger, R., Rotter, J.I., Lipton, R.B., Bullido, M.J., Hofman, A., Montine, T.J., Coto, E., Boerwinkle, E., Petersen, R.C., Alvarez, V., Rivadeneira, F., Reiman, E.M., Gallo, M., O'Donnell, C.J., Reisch, J.S., Bruni, A.C., Royall, D.R., Dichgans, M., Sano, M., Galimberti, D., St George-Hyslop, P., Scarpini, E., Tsuang, D.W., Mancuso, M., Bonuccelli, U., Winslow, A.R., Daniele, A., Wu, C.K., GERAD/PERADES, CHARGE, ADGC, EADI, Peters, O., Nacmias, B., Riemenschneider, M., Heun, R., Brayne, C., Rubinsztein, D.C., Bras, J., Guerreiro, R., Al-Chalabi, A., Shaw, C.E., Collinge, J., Mann, D., Tsolaki, M., Clarimon, J., Sussams, R., Lovestone, S., O'Donovan, M.C., Owen, M.J., Behrens, T.W., Mead, S., Goate, A.M., Uitterlinden, A.G., Holmes, C., Cruchaga, C., Ingelsson, M., Bennett, D.A., Powell, J., Golde, T.E., Graff, C., De Jager, P.L., Morgan, K., Ertekin-Taner, N., Combarros, O., Psaty, B.M., Passmore, P., Younkin, S.G., Berr, C., Gudnason, V., Rujescu, D., Dickson, D.W., Dartigues, J.F., DeStefano, A.L., Ortega-Cubero, S., Hakonarson, H., Campion, D., Boada, M., Kauwe, J.K., Farrer, L.A., Van Broeckhoven, C., Ikram, M.A., Jones, L., Haines, J.L., Tzourio, C., Launer, L.J., Escott-Price, V., Mayeux, R., Deleuze, J.F., Amin, N., Holmans, P.A., Pericak-Vance, M.A., Amouyel, P., van Duijn, C.M., Ramirez, A., Wang, L.S., Lambert, J.C., Seshadri, S., Williams, J., Schellenberg, G.D., 2017. Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. Nat.Genet. 49, 1373-1384.

Slattery, C.F., Beck, J.A., Harper, L., Adamson, G., Abdi, Z., Uphill, J., Campbell, T., Druyeh, R., Mahoney, C.J., Rohrer, J.D., Kenny, J., Lowe, J., Leung, K.K., Barnes, J., Clegg, S.L., Blair, M., Nicholas, J.M., Guerreiro, R.J., Rowe, J.B., Ponto, C., Zerr, I., Kretzschmar, H., Gambetti, P., Crutch, S.J., Warren, J.D., Rossor, M.N., Fox, N.C., Collinge, J., Schott, J.M., Mead, S., 2014. R47H TREM2 variant increases risk of typical early-onset Alzheimer's disease but not of prion or frontotemporal dementia. Alzheimers Dement. 10, 602-608.e4.

Strandberg, T.E., Salomaa, V., Strandberg, A.Y., Vanhanen, H., Sarna, S., Pitkala, K., Rantanen, K., Savela, S., Pienimaki, T., Huohvanainen, E., Stenholm, S., Raikkonen, K., Tilvis, R.S., Tienari, P.J., Huttunen, J., 2016. Cohort Profile: The Helsinki Businessmen Study (HBS). Int.J.Epidemiol. 45, 1074-1074h.

Uusvaara, J., Pitkala, K.H., Kautiainen, H., Tilvis, R.S., Strandberg, T.E., 2013. Detailed cognitive function and use of drugs with anticholinergic properties in older people: a community-based cross-sectional study. Drugs Aging. 30, 177-182.

Yliharsila, H., Kajantie, E., Osmond, C., Forsen, T., Barker, D.J., Eriksson, J.G., 2007. Birth size, adult body composition and muscle strength in later life. Int.J.Obes.(Lond). 31, 1392-1399.

Zhang, B., Gaiteri, C., Bodea, L.G., Wang, Z., McElwee, J., Podtelezhnikov, A.A., Zhang, C., Xie, T., Tran, L., Dobrin, R., Fluder, E., Clurman, B., Melquist, S., Narayanan, M., Suver, C., Shah, H., Mahajan, M., Gillis, T., Mysore, J., MacDonald, M.E., Lamb, J.R., Bennett, D.A., Molony, C., Stone, D.J., Gudnason, V., Myers, A.J., Schadt, E.E., Neumann, H., Zhu, J., Emilsson, V., 2013. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. Cell. 153, 707-720.

	HBCS	HBS	VV85+	DEBATE	Total
Total (n)	8760	3490	553	4821	17624
DNA available (n)	1680	672	531	377	3260
Successfully genotyped	1678	646	526	370	3220
Number of deletions (%)	6 (0.36)	3 (0.46)	2 (0.38)	0	11 (0.34)
MMSE available (n)	898	609	513	370	2390
mean age	68,1	75,7	88,5	80,1	76,3
SD of age	4,8	3,9	2,84	4,8	8,5
men (%)	40	100	20	65	55
MMSE/other info available (n)	1678	619	525	370	3192
mean age	71,7	84,3	88,5	88,6	79
SD of age	3,2	4,9	2,84	4,8	8,6
men (%)	44	100	21	65	53

Supplementary Table 1: Cohort information. First four rows show the number of individuals in the four cohort studies, the number of individuals used in this study and the number of deletions found. The fifth row shows the number of individuals whose MMSE scores were available. The ninth row shows the number of individuals from which cognitive impairment status was known either from MMSE scores or other information available.

	Vantaa 85+ study		Helsinki businessmen study			Helsinki birth cohort study					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Age at MMSE	89	85	74	79	82	68	68	-	-	-	-
MMSE score	27	21	25	29	27	29	28	-	-	-	-
Age at additional information	89 (clinical examination) 90 (autopsy)	85 (clinical examination) 94 at autopsy	85	89	91	73	73	69	70	69	60
Additional information on cognition	No clinical Alzheimer's disease Braak stage 3 CERAD score 0 Neocortical B-amyloid 0%	No clinical Alzheimer's disease Braak stage 4 CERAD score 0 Neocortical B-amyloid 0.18%	No dementia	Alzheimer's disease and vascular dementia	Alzheimer's disease	No dementia	No dementia	No dementia	No dementia. Died at 70 of colorectal cancer	No dementia	No dementia. Died at 60 of coronary disease and pneumonia
Sex	female	female	male	male	male	female	female	male	female	male	female
***APOE* e4**	2/3	2/3	3/4	2/3	3/3	3/4	3/3	3/4	3/4	3/3	3/4

Supplementary Table 2. A summary of the characteristics of the 11 deletion carriers